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Pharmacokinetic/Pharmacodynamic Predictors of Clinical Potency for Hepatitis C Virus Nonnucleoside Polymerase and Protease Inhibitors

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This analysis was conducted to determine whether the hepatitis C virus (HCV) viral kinetics (VK) model can predict viral load (VL) decreases for nonnucleoside polymerase inhibitors (NNPolIs) and protease inhibitors (PIs) after 3-day monotherapy studies of patients infected with genotype 1 chronic HCV. This analysis includes data for 8 NNPolIs and 14 PIs, including VL decreases from 3-day monotherapy, total plasma trough concentrations on day 3 (C_{min}), replicon data (50% effective concentration [EC_{50}] and protein-shifted EC_{50} [$EC_{50,PS}$]), and for PIs, liver-to-plasma ratios (LPRs) measured *in vivo* in preclinical species. VK model simulations suggested that achieving additional log_{10} VL decreases greater than one required 10-fold increases in the C_{min}. NNPolI and PI data further supported this result. The VK model was successfully used to predict VL decreases in 3-day monotherapy for NNPolIs based on the $EC_{50,PS}$ and the day 3 C_{min}. For PIs, however, predicting VL decreases using the same model and the $EC_{50,PS}$ and day 3 C_{min} was not successful; a model including LPR values and the EC_{50} instead of the $EC_{50,PS}$ provided a better prediction of VL decrease. These results are useful for designing phase 1 monotherapy studies for NNPolIs and PIs by clarifying factors driving VL decreases, such as the day 3 C_{min} and the $EC_{50,PS}$ for NNPolIs or the EC_{50} and LPR for PIs. This work provides a framework for understanding the pharmacokinetic/pharmacodynamic relationship for other HCV drug classes. The availability of mechanistic data on processes driving the target concentration, such as liver uptake transporters, should help to improve the predictive power of the approach.

The introduction of direct-acting antiviral (DAA) drugs with activity against the hepatitis C virus (HCV) is expected to significantly reduce the growing impact of this global epidemic, providing better treatment options to many patients and reducing the economic burden of disease. To realize the full potential of these emerging therapies, it is important to have an understanding of those factors which directly impact the clinical efficacy of these drugs. One important factor is the relationship between pharmacokinetics and pharmacodynamics (PK/PD), which aims to provide the link between a dosage regimen and a clinical outcome. For antiviral activity, this link requires knowledge of the concentration of drug at the site of action and the susceptibility of the virus to the particular drug of interest. Understanding the PK/PD of a particular drug or drug class then allows for the optimization of dosing regimens in clinical practice, enhances the ability to select the most appropriate doses during the drug development process, and helps guide the design and selection of future drug candidates.

The FDA recommends that in phase 1b studies in HCV patients, doses be selected so that the total plasma trough concentration (C_{min}) is higher than the protein-shifted 50% effective concentration ($EC_{50,PS}$) by severalfold (12). Although longer monotherapy studies are permitted for inhibitors with high barriers to resistance (e.g., the ELECTRON trial was amended to include one arm exploring 12 weeks of PSI-7977 monotherapy), for compounds with a low barrier to resistance, monotherapy longer than 3 days is not recommended due to the potential emergence of resistant viral strains. Three days should be sufficient for an initial exploration of the potential antiviral activity of a range of doses (12). The work presented here is aimed at understanding how to predict the PK/PD response after 3-day monotherapy to assist in the design of this proof-of-concept study for HCV nonnucleoside polymerase inhibitors (NNPolIs) and protease inhibitors (PIs) in early development.

A viral kinetics (VK) model describing the viral titers in HCV patients and their decline under therapy (e.g., with interferon alpha-2b) has been developed (50). The value of this approach has been demonstrated by many, e.g., in optimizing the design of HCV VK studies (18), in predicting the time required to reach undetectable viral load (VL) based on the observed 14-day VL (22), and in exploring potential mechanisms related to success of treatment and viral breakthrough during therapy (66), to name only a few demonstrated applications. Recently the VK model has been refined using the population approach with extensive clinical data in chronic HCV patients receiving peginterferon alpha-2a with or without ribavirin coadministration, with the added feature of a viral eradication boundary so that the percentage of patients achieving a cure can be simulated (66). The analysis described here was conducted to determine whether this refined HCV VK model could be used to predict VL decreases for NNPolIs and PIs in a 3-day monotherapy study with chronic HCV patients infected with genotype 1 (GT-1) based on *in vitro* replicon potency data and other preclinical data. Monotherapy and potency data for 8 NNPolIs and for 14 PIs were used in the analysis. As the FDA recommends guiding phase 2 study design using a mechanism-based VK model developed from phase 1 data (12), this analysis leverages that approach with even earlier application.

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TABLE 1 Summary of NNPolI 3-day monotherapy studies in HCV GT-1 patients

Compound	Dose (mg)	Regimen[i]	$N/N_{1a}/N_{1b}$[a]	Day 3 C_{min} (μg/ml)[b]	Day 3 VL decrease (log_{10} IU/ml[c])		Reference(s)
					Observed	Predicted[d]	
ABT-072	100	QD	8/5/3	0.050	−1.14	−1.1	11, 15
	300	QD	8[h]/5/2	0.15	−1.07	−1.5	
	600	QD	7/6/1	0.30	−1.57	−1.7	
ABT-333	400	BID	8/5/3	0.51	−1.08	−1.3	15, 26, 45
	800	BID	8/6/2	0.51	−0.95	−1.3	
Filibuvir, PF-00868554	100	BID	6	0.551	−0.70	−1.0	19
	300	BID	6	0.994	—[e]	—[e]	
	450	BID	6	0.868	—[e]	—[e]	
	300	TID	6	1.13	−1.8	−1.3	
IDX375	100	BID	Not reported	3.4	−1.3	−2.2	9, 21
	200	BID	Not reported	6.8	−2.3	−2.5	
	400	BID	Not reported	14	−2.7	−2.8	
MK-3281	800	BID	11/7[g]/4	1.63	−0.80/−2.9[f]	−1.6/−1.6[f]	2
Setrobuvir, ANA598	200	BID	11/5/6	35	−1.43/−2.55[f]	−1.9/−3.1[f]	35
	400	BID	8/3/5	75	−1.79/−2.50[f]	−2.2/−3.4[f]	
	800	BID	8/4/4	190	−2.50/−3.23[f]	−2.6/−3.8[f]	
Tegobuvir, GS-9190	40	BID	11/8/3	0.62	−1.0	−1.2	1
	120	BID	12/10/2	2.5	−1.5	−1.8	
VCH-222	750	BID	5/4/1	2.1	−3.7	−1.5	7

[a] N, total number of patients; N_{1a}, and N_{1b}, number of GT-1a and GT-1b patients in a dose group. When only one number was reported, N_{1a} and N_{1b} were not available.
[b] When not reported, C_{min} values were calculated as described in Materials and Methods and the Appendix.
[c] Units for tegobuvir were copies/ml.
[d] Predicted using the VK model using the $C_{min}/EC_{50,PS}$ ratio to estimate the PD effect.
[e] Because emergence of resistant strains appeared to be affecting the two middle-dose groups, these values were left out of the table and the results plot since they are easy to misinterpret.
[f] VL decreases (observed and predicted) for patients with GT-1a and GT-1b are listed separately (GT-1a/GT-1b) if the information was reported and there were >3 subjects for each.
[g] This group included GT-1a and GT-NT (nontypeable).
[h] One subject was GT-NT.
[i] QD, once daily; BID, twice daily; TID, three times a day.

MATERIALS AND METHODS

NNPolI and PI GT-1 monotherapy study data. Data for VL decreases in chronic HCV patients infected with GT-1 and treated in monotherapy for at least 3 days were obtained for 8 NNPolIs (Table 1) and for 14 PIs (Table 2). Here, monotherapy refers to DAA administered without interferon or ribavirin; two of the PIs (i.e., ABT-450 and narlaprevir) were administered with ritonavir as a PK enhancer. *In vitro* potency in the HCV replicon system has been reported for each compound. When available, potency (with 5 to 10% fetal bovine serum) for both GT 1a and GT 1b replicons, as well as protein-shifted potency (with 40 to 50% human serum added), were used in this analysis (Table 3). For PIs, the liver-to-plasma ratio (LPR) measured *in vivo* in a variety of preclinical species was also used (Table 4).

The monotherapy data presented here are from a variety of study designs in terms of doses, durations, patient populations, and HCV genotypes. Some of the studies were 3-day monotherapy studies, but more often the studies were longer monotherapy studies or included a period of monotherapy prior to a period of coadministration with pegylated interferon alpha-2a and ribavirin. From each study, only data up to day 3 of monotherapy in patients with GT-1a and GT-1b were included.

HCV viral titers can be measured with several different assays, and there can be differences in the reported VL depending on the assay used (27, 52). But regardless of the assay or whether a VL is reported in IU/ml or copies/ml, a 1-log_{10} decrease from baseline is a 10-fold decrease in VL. Therefore, the model prediction of VL drop could be compared to the experimental data regardless of the assay used to measure HCV viral titers.

This analysis used VL decrease data on day 3 of monotherapy, with some exceptions. For telaprevir, danoprevir, and the two lower TMC435350 doses, the data were on day 2 of monotherapy because day 3 values were not reported. However, for two of those compounds (i.e., telaprevir and danoprevir), the model underestimated the log_{10} VL decreases. The prediction would have looked more accurate without including these compounds. Also, the VL decrease is most rapid at times less than 2 days and then slows down, as illustrated here with VK model simulations (Fig. 1) and also demonstrated in monotherapy studies, e.g., for danoprevir (13). Therefore, the 3-day time point would probably not have been that much lower, and it was appropriate to include these compounds in the analysis.

In monotherapy study reports, mean VL decrease and PK data were most often reported together. When mean and median VL data were both provided, median data were used in this analysis due to the high variability often observed in VK data. For setrobuvir, danoprevir, GS-9256, and telaprevir, as well as the high dose of TMC435350, median VL decrease data were reported. For VCH-222 and MK-5172, individual values were reported and the median was computed. For filibuvir, tegobuvir, MK-3281, and VCH-222, day 3 VL decrease values were read from graphed data. The procedure for reading data off plots was to capture the image using Snag It version 9.1.3 (TechSmith, Tokyo, Japan) and then extract the values from

TABLE 2 Summary of PI 3-day monotherapy studies in HCV GT-1 patients

Compound (notes on study)	Dose (mg)	Regimen[a]	$N/N_{1a}/N_{1b}$[b]	Day 3 C_{min} (μg/ml)[c]	Day 3 VL decrease (log_{10} IU/ml)		Reference(s)
					Observed	Predicted[d]	
ABT-450 with 100 mg ritonavir	50	QD	8/7/1	0.005	−4.07	−1.9	32
	100	QD	8/5/3	0.017	−3.89	−2.5	
	200	QD	8/7/1	0.082	−4.11	−3.1	
ACH-1625 (2nd 600-mg group was fed)	400	QD	6	0.0759	−3.2	−3.8	10
	600	QD	6	0.0311	−3.3	−3.4	
	600	QD	6	0.0766	−3.6	−3.8	
Asunaprevir, BMS-650032	200	BID	4/3/1	0.027	−3.2	−2.8	41, 51
	400	BID	4/4/0	0.054	−3.4	−3.0	
	600	BID	4/3/1	0.081	−2.6	−3.3	
BI 201335	20	QD	6/3/3	0.063	−2.9	−3.0	42
	48	QD	7/2/5	0.19	−3.5	−3.5	
	120	QD	7/2/3	0.98	−3.6	−4.2	
	240	QD	6/3/2	3.5	−4.0	−4.6	
Boceprevir, SCH 503034	100	BID	12	0.011	−0.3	−0.26	65, 74
	200	BID	12	0.022	−0.5	−0.43	
	400	BID	11	0.048	−0.6	−0.68	
	400	TID	10	0.19	−1.7	−1.2	
Danoprevir (1st 4 groups were TN, but 300-mg BID group were NRs[f])	100	BID	8/4/4	0.00003	−1.1[e]	−0.73	13
	100	TID	8/2/6	0.00024	−2.1[e]	−1.6	
	200	BID	5/1/4	0.00020	−2.6[e]	−1.5	
	200	TID	8/4/4	0.00101	−3.9[e]	−2.2	
	300	BID	8/3/5	0.00043	−2.3[e]	−1.8	
GS-9256 (1st 3 groups received solution formulation, last 2 got capsules)	75	BID	12/6/6	1.9	−2.6		34
	200	BID	8/6/2	21.6	−2.9		
	300	QD	7/6/1	3.2	−2.6		
	25	BID	9/8/1	0.2	−1.1		
	75	BID	9/8/1	1.3	−2.7		
GS-9451	60	QD	8/8/0	0.050	−0.88		33
	200	QD	8/8/0	0.65	−3.2		
	400	QD	9/9/0	1.86	−3.6		
	200	QD	8/1/7	0.53	−3.5		
IDX320	50	QD	5/0/5	0.065	−2.5	−3.1	59
	100	QD	6/0/6	0.118	−3.1	−3.3	
	200	QD	5/0/5	0.174	−3.1	−3.5	
	400	QD	6/0/6	0.274	−3.3	−3.7	
	200	BID	6/0/6	0.781	−3.8	−4.1	
MK-5172	400	QD	5	0.0928[g]	−3.8		55
Narlaprevir, SCH 900518 (400 mg BID dose coadministered with 200 mg ritonavir)	800	TID	TN, 10/4/5	0.31	−4.05		58
	400	BID	TN, 10/4/4	1.7	−4.0		
	800	TID	TE, 10/1/5	0.31	−3.95		
	400	BID	TE, 10/3/6	1.7	−3.55		
Telaprevir, VX-950	450	TID	10	0.78	−3.11[e]	−1.9	61
	750	TID	8	1.1	−3.35[e]	−2.0	
	1,250	BID	8	0.68	−2.89[e]	−1.8	
TMC435350	200	QD	6/4/2	4.61	−3.6	−4.9	43, 60
	25	QD	9	0.071	−2.7[e]	−3.3	
	75	QD	10	0.27	−3.1[e]	−3.8	

(Continued on following page)

TABLE 2 (Continued)

Compound (notes on study)	Dose (mg)	Regimen[a]	$N/N_{1a}/N_{1b}$[b]	Day 3 C_{min} (μg/ml)[c]	Day 3 VL decrease (log_{10} IU/ml)		Reference(s)
					Observed	Predicted[d]	
Vaniprevir, MK-7009	25	BID	3/1/1	0.00056	−2.1	−2.3	36, 73
	75	BID	6/5/0	0.0035	−3.2	−3.5	
	250	BID	6/5/1	0.014	−3.4	−3.9	
	500	BID	5/4/0	0.025	−3.6	−4.3	
	125	QD	5/2/2	0.00039	−3.1	−2.2	
	600	QD	4/4/0	0.0035	−3.4	−3.5	
	700	BID	6/5/1	0.044	−4.05	−4.4	

[a] QD, once daily; BID, twice daily; TID, three times a day.
[b] N, total number of patients in each group being administered drug; N_{1a} and N_{1b}, the number of patients was broken down into the number of GT-1a and GT-1b patients when possible—when N_{1a} and N_{1b} do not add up to the total number, there were subjects where GT-1a or GT-1b could not be determined; when only one number was reported, N_{1a} and N_{1b} were not available; TN, treatment naïve; TE, treatment experienced.
[c] C_{min} values were sometimes reported but sometimes calculated as described in Materials and Methods and the Appendix.
[d] Calculated using the most predictive PK/PD index, $C_{min} \times LPR/EC_{50}$, as input for the model.
[e] These data were from day 2.
[f] NR, nonresponder.
[g] This value was calculated from a reported C_{min} of 121 nM. Although the units in the monotherapy study were reported as μM (55), nM was determined to be correct by comparing the C_{min} with the C_{max} and by comparing C_{min} in healthy volunteers with C_{min} from another study (54).

the images. For all PIs except ABT-450 and MK-5172, VL decreases were read off plots. For GS-9451, median maximum VL decreases were provided from a 3-day monotherapy study, and those values were used instead of reading the day 3 values from the plot because they occurred at or near day 3. In the case of ABT-072 and ABT-333, mean maximum VL decreases were provided from a 3-day monotherapy study, and those values were used instead of reading the day 3 values from plots because the maximum VL decrease appeared to take place on day 3.

PK data were read off plots for tegobuvir, setrobuvir, ABT-072, and VCH-222. For ABT-072 and vaniprevir, PK data in HCV patients were limited or not reported, and the C_{min} values used here were from studies in healthy volunteers. For boceprevir, C_{min} data were not reported in the monotherapy studies with GT-1; C_{min} data from a study on monotherapy in patients with HCV genotypes 2 and 3 (65) were used instead. Additional details on calculations required for specific compounds (e.g., how repeat-dose C_{min} was estimated from single-dose PK data when necessary) are located in the Appendix.

For PIs, LPR data were reported for a variety of species (Table 4). The LPR is typically determined in PK studies as either the measured total liver concentration divided by the total plasma concentration at a time point or as the ratio of area under the concentration-time curve (AUC) values for the total concentrations in the two tissues. In this analysis, it was assumed that the LPR was a constant value over time, independent of plasma concentrations (i.e., that uptake by liver transporters was not saturated), and that the human LPR could be approximated using the values determined in preclinical species (i.e., that the species difference in LPRs would be minimal). Although large species differences could exist, the measured LPR values in preclinical species were the only data available for incorporating this important consideration in the model. For danoprevir, calculations including LPR used the value in monkeys (127) instead of the value in rats (10) because the plasma concentrations were about 1 to 2 orders of magnitude lower in the monkey study and so, in the rat study, liver uptake transporters might have been saturated. For telaprevir, the average LPR between mice and rats (23) was used. For ACH-1625, the LPR in rats (505) was used because for dogs, it was reported as >55. For asunaprevir, a value of 40 was used since it was reported as ≥40 across species.

Replicon data. When VL decreases were reported for GT-1 but not for GT-1a and GT-1b separately, an EC_{50} reflective of the proportion of each GT in a given dose group was used in the calculations, as follows:

$$EC_{50} = \left(N_{1a} \times EC_{50,1a} + N_{1b} \times EC_{50,1b}\right) / \left(N_{1a} + N_{1b}\right) \quad (1)$$

where N_{1a} and N_{1b} are the number of GT-1a and GT-1b patients in a dose group, respectively, and $EC_{50,1a}$ and $EC_{50,1b}$ are the replicon EC_{50}s for GT-1a and GT-1b, respectively. A similar method was used to estimate appropriate values for the $EC_{50,PS}$. When N_{1a} and N_{1b} were not reported (e.g., as was the case for telaprevir), the average of the GT-1a and GT-1b EC_{50} (or $EC_{50,PS}$) was used. If replicon data for only one GT were available, then that EC_{50} was used in calculations. If multiple values were available, a mean value was used unless otherwise noted. It should be noted that the replicon data were generated in different laboratories and may reflect slight variations in each assay (e.g., duration of assay).

Viral kinetics model. The VK model of Snoeck et al. (66) was used for this analysis. Briefly, the model describes the infection of target cells (T, hepatocytes/ml) by HCV virions (V, virions/ml) to form infected cells (I, hepatocytes/ml), as follows:

$$\frac{dT}{dt} = s + r \cdot T \cdot \left(1 - \frac{T+I}{T_{max}}\right) - d \cdot T - \beta \cdot V \cdot T \quad (2)$$

$$\frac{dI}{dt} = \beta \cdot V \cdot T + r \cdot I \cdot \left(1 - \frac{T+I}{T_{max}}\right) - \delta \cdot I \quad (3)$$

$$\frac{dV}{dt} = (1-\varepsilon) \cdot p \cdot I - c \cdot V \quad (4)$$

where t is time, s is the hepatocyte production rate, r is the hepatocyte proliferation rate constant in both target and infected cells, T_{max} is the total number of hepatocytes per ml, d is the hepatocyte death rate constant, β is the second-order rate constant for target cell infection, δ is the infected hepatocyte death rate constant, p is the virion production rate constant, c is the virion elimination rate constant, and ε is the inhibition caused by the NNPolI or PI described using a maximum effect (E_{max}) model assuming a maximum inhibition of 100%. The original model contained both infectious and noninfectious virions due to the effect of ribavirin, but equations 2 and 4 were written to describe VK during monotherapy, and therefore, only infectious virions were included. In this analysis, it was assumed that the inhibition from NNPolIs and PIs reduced viral production, i.e., both were incorporated in the model the same way. For NNPolIs and for PIs, the following method of parameterizing the E_{max} model was explored to determine whether it could predict VL decreases based on the inhibition at the day 3 total plasma trough concentration, C_{min}:

$$\varepsilon = \frac{C_{min}/EC_{50,PS}}{C_{min}/EC_{50,PS} + 1} \quad (5)$$

TABLE 3 Summary of selected properties and potency for NNPolIs and PIs

Target and compound (site for NNPolIs)	MW[a]	EC_{50} (nM)[b]		$EC_{50,PS}$ (nM)[b]		Reference(s)
		GT-1a	GT-1b	GT-1a	GT-1b	
NNPolIs						
ABT-072 (palm 1)		1.1	0.3	20	2.5	71
ABT-333 (palm 1)		8	2	100	20	71
Filibuvir (thumb 2)	503.6	52	33	190	120	71
IDX375 (palm 1)			2.3		60	71
MK-3281 (thumb 1)	475.6	40	40	110	110	71
Setrobuvir (palm 1)		52	3	1,400[c]	78[c]	71
Tegobuvir (thumb)	517.4	3.6	1.2	66[d]	22[d]	71
		13.8	0.8	250[d]	15[d]	64
		3.6	0.6	66[d]	11	1a
VCH-222 (thumb 2)		22	11	210	105	71
PIs						
ABT-450		0.9	0.3	19	7	24
ACH-1625		15	11	50[d]	37	20
Asunaprevir	748.3	4	1			44
BI 201335	869.8	6.5	3.1			72
Boceprevir	519.7		480		1,180	40, 72
		550	520	1,350[d]	1,280[d]	
Danoprevir	731.8		1.8[e]			24, 63
		2.2[e]	1.3[e]	40[e]	18[e]	
GS-9256	957.5	74	20			46
GS-9451	910.5	22	7.5			8
IDX320		0.5	3.4			30
MK-5172	766.0[f]		2		9.5	54
Narlaprevir	708.0		20			69
Telaprevir	679.8		1,100		8,600	20, 40, 72
		700	540	4,460[d]	3,440[d]	
		213	408	1,360[d]	3,600	
TMC435350	749.9	28.4	8.1	49[d]	14.1	38
Vaniprevir	757.9	0.75	0.9	29	21	24, 40
			5		13	

[a] For compounds with an unknown molecular weight (MW), the average value of all the others for that target was used in calculations, i.e., 500 g/mol for NNPolIs and 760 g/mol for PIs.
[b] EC_{50} is from a replicon assay with 5 to 10% fetal bovine serum, and $EC_{50,PS}$ is from a replicon assay with 40 to 50% human serum added, i.e., a protein-shifted assay.
[c] Not measured experimentally. Calculated based on the observed protein-shift with 40% human serum of 26 (68).
[d] Not measured experimentally. Calculated assuming the protein-shift observed for GT-1b.
[e] Data from reference 24 were used because more data were available and GT-1b EC_{50}s were consistent.
[f] The MW of the potassium salt is 805.0.

TABLE 4 Liver-to-plasma ratio (LPR) data for PIs in various preclinical species

PI	LPR(s), species	Reference
ABT-450	6.7, dog	24
ABT-450 with ritonavir	7.0, dog	24
ACH-1625	>100 i.v. and 505 oral, rat[a]; >55, dog	67
Asunaprevir	≥40 across species	44
BI 201335	42, rat	72
Boceprevir	30, rat	5
Danoprevir	10, rat; 127, monkey	14
IDX320	32, mouse	17
Telaprevir	5.7-16, mouse; 35, rat	53
TMC435350	39, rat	38
Vaniprevir	330, chimpanzee[b]	40

[a] For this analysis, an LPR value of 505 was used. i.v., intravenous.
[b] Estimated using 12-h plasma and liver concentrations read off plots.

Since the $EC_{50,PS}$ is from an assay with protein present, it was reasonable to compare total C_{min} to this value for the antiviral activity prediction. For PIs, three additional ways to parameterize the E_{max} model were explored:

$$\varepsilon = \frac{C_{min}/EC_{50}}{C_{min}/EC_{50} + 1} \quad (6)$$

$$\varepsilon = \frac{C_{min} \times LPR/EC_{50,PS}}{C_{min} \times LPR/EC_{50,PS} + 1} \quad (7)$$

$$\varepsilon = \frac{C_{min} \times LPR/EC_{50}}{C_{min} \times LPR/EC_{50} + 1} \quad (8)$$

Regardless of whether EC_{50} or $EC_{50,PS}$ was used as the model input, C_{min} in these equations is the total trough concentration on day 3. Equations 7 and 8 are based on the assumption that the effect of liver uptake transporters, approximated using the LPR measured in preclinical species, increases the free-drug concentration in the liver and thereby increases target inhibition. Additionally, equations 5 through 8 were written assuming that C_{min} was the PK parameter responsible for antiviral activity, which was assumed for reasons presented in Discussion. Initially, a Hill coefficient (i.e., a sigmoidal E_{max} model) was explored as one possible way to improve the prediction of the day 3 VL decrease. However, it added another model parameter and did not improve the predictions. Therefore, no Hill coefficient was included in this analysis.

This analysis did not consider interindividual variability since individual data were unavailable. Instead simulations used the typical parameter values reported by Snoeck et al. (66), which were derived using a population modeling approach with an extensive data set from one phase 2 study and four phase 3 studies with chronic HCV patients who received treatment with peginterferon alpha-2a with or without ribavirin. The model development included patients with GT-1 and other genotypes, but we used model parameters for GT-1. The model was implemented in Berkeley Madonna version 8.3.11 (University of California at Berkeley, Berkeley, CA), a software package for solving systems of ordinary differential equations. Plots of the VL decrease from baseline on day 3 as a function of PK/PD indices were simulated in Berkeley Madonna using a parameter plot, which is a convenient way to tabulate the final value of multiple simulations for a parameter value changing over a specified range.

RESULTS

VK model simulations. Simulated $\log_{10}$ VL decreases from baseline as a function of time from 3 days of monotherapy in chronic HCV GT-1-infected patients are shown in Fig. 1. These results indicate that for a compound to achieve a 1-$\log_{10}$ de-

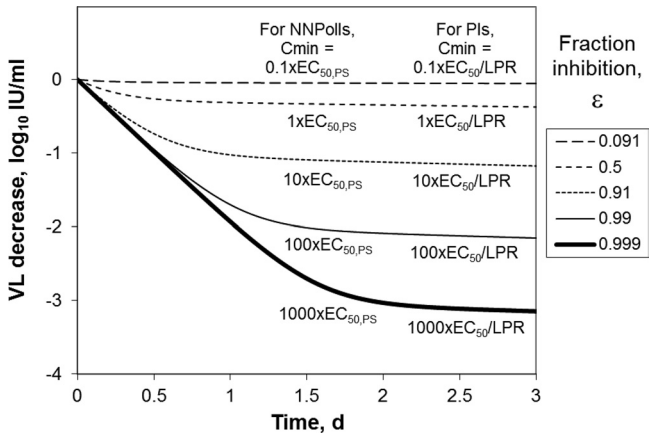

FIG 1 Simulated VL decrease from baseline as a function of time in monotherapy of patients infected with HCV GT-1 for different levels of inhibition.

TABLE 5 Summary of selected NNPolI and PI results

Compound	HD (mg) and regimen[a]	HD day 3 $\log_{10}$ VL decrease	HD day 3 C_{min}/EC_{50}	HD day 3 $C_{min}/EC_{50,PS}$	HD day 3 $C_{min} \times LPR/EC_{50}$	HD day 3 $C_{min} \times LPR/EC_{50,PS}$	C_{min} range[b]
NNPolIs							
ABT-072	600 QD	−1.6	610	34			6.0-fold
ABT-333[c]	400–800 BID[c]	−1.1	180	15			NA[c]
Filibuvir	300 TID	−1.8	53	14			2.1-fold
IDX375	400 BID	−2.7	12,000	450			4.0-fold
MK-3281	800 BID	−0.80/−2.9[d]	86/86[d]	31/31[d]			NA
Setrobuvir	800 BID	−2.5/−3.2[d]	7,100/120,000[d]	270/4,800[d]			5.3-fold
Tegobuvir	120 BID	−1.5	800	44			4.0-fold
VCH-222	750 BID	−3.7	210	22			NA
PIs							
ABT-450 with ritonavir[e]	50–200/100 QD	−3.9 to −4.1	7.9–130	0.37–6.1	55–910	2.6–43	16-fold
ACH-1625	600 QD fed	−3.6	7.7	2.3	3,900	1,200	2.5-fold
Asunaprevir	600 BID	−3.4[f]	33		1,300		1.5-fold
BI 201335	240 QD	−4.0	770		32,000		55-fold
Boceprevir	400 TID	−1.7	0.35	0.071	10	2.1	17-fold
Danoprevir[g]	200 TID	−3.9	0.79	0.048	100	6.0	34-fold
GS-9256	200 BID solution	−2.9	370				110-fold
GS-9451	400/200 QD[d]	−3.6/−3.5[d]	93/78[d]				11-fold
IDX320	200 BID	−3.8	300		9,700		12-fold
MK-5172	400 QD	−3.8	61	13			NA
Narlaprevir[h]	800 TID	−4.1	22		86		NA
Narlaprevir with ritonavir[h]	400/200 BID	−4.0	120		480		NA
Telaprevir[g]	750 TID	−3.4	2.7	0.33	63	7.6	1.6-fold
TMC435350	200 QD	−3.6	280	160	11,000	6,400	65-fold
Vaniprevir	700 BID	−4.1	52	2.1	17,000	710	>33-fold

[a] HD, high dose; QD, once daily; BID, twice daily; TID, three times a day. The high dose is considered to be the dose resulting in the highest C_{min}; e.g., 200 mg TID would be considered a higher dose than 300 mg BID if it resulted in a higher C_{min}.
[b] C_{min} range, reported as fold difference, is the HD C_{min} divided by the C_{min} at the lowest dose. NA, not applicable, is reported for studies that only included one dose level.
[c] The C_{min} and $\log_{10}$ VL decreases were similar for the 400 and 800 mg BID dose groups. Dose-limited absorption led to similar C_{min} values at both doses. Here, the data are for the 400-mg BID dose group, which had a slightly higher VL drop.
[d] Data or calculation for GT-1a/GT-1b, which were treated separately.
[e] The VL decrease was independent of dose; the range reported is for the low to the high dose.
[f] VL decrease is for second highest dose; HD had the lowest VL decrease, which seems spurious.
[g] Most data are from day 3; for telaprevir and danoprevir, data are from day 2.
[h] Data are for treatment-naïve instead of treatment-experienced groups because C_{min} values and VL decreases for treatment-naïve patients were higher. The group with ritonavir was reported separately since it might affect the PK/PD relationship.

crease in VL over 3 days, the inhibition of replication, ε, must be maintained at more than 90%. Figure 1 also lists the C_{min} relative to the potency required for NNPolIs and PIs, as discussed further below. For both NNPolIs and protease inhibitors, each 10-fold increase in C_{min} is expected to result in an additional VL decrease of about 1 $\log_{10}$.

In reports of monotherapy results, the authors have sometimes concluded that saturation of response was observed, e.g., for PI IDX320 (59). Therefore, this result, that additional 10-fold increases in C_{min} will result in additional $\log_{10}$ VL decreases, requires more investigation. Next, observed clinical data were used to assess this prediction.

PK/PD relationship for NNPolIs. The NNPolIs included in this analysis, for which selected clinical data are summarized in Tables 1 and 5, achieved decreases of up to about 3.7-$\log_{10}$ IU/ml in VL from baseline in 3 days of monotherapy (i.e., for VCH-222), but for half the NNPolIs included here, the VL decreases were in the range of 1 to 2 $\log_{10}$ IU/ml. At the high dose in monotherapy studies, the day 3 total $C_{min}/EC_{50,PS}$ ranged from 14 for filibuvir (VL decrease from baseline of 1.8 $\log_{10}$ IU/ml) to 4,800 for setrobuvir in GT-1b patients (VL decrease from baseline of 3.2 $\log_{10}$ IU/ml) (Table 5). Predicted $\log_{10}$ VL decreases from 3 days of NNPolI monotherapy in patients with GT-1 as a function of day 3 $C_{min}/EC_{50,PS}$ are compared to observed data in Fig. 2. Plotting the data in this normalized way allows the comparison of simulation results with data for multiple NNPolIs. Although most individual studies did not cover a wide enough range of C_{min} to fully characterize the dose-response relationship, by combining data for multiple compounds, the relationship is clarified.

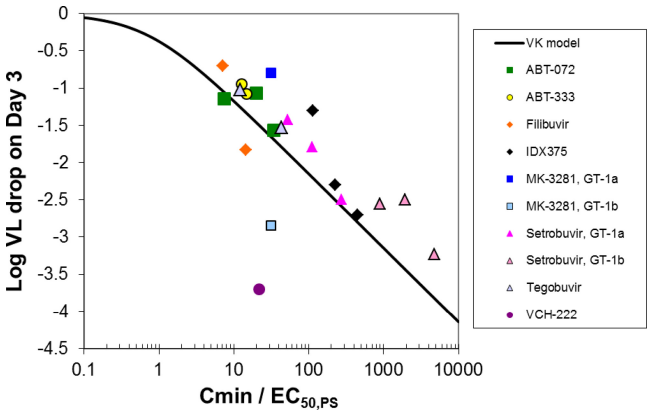

FIG 2 Predicted (line) and observed (symbols) VL decrease from baseline on day 3 of NNPolI monotherapy ($\log_{10}$ IU/ml) as a function of $C_{min}/EC_{50,PS}$.

In general, the simulation results are consistent with the observed data for NNPolIs, with a tendency to overestimate VL decreases. The overestimation could be due to the emergence of resistant virus, which can be selected very readily upon treatment due to their presence at very low levels in the patient's viral population prior to the initiation of treatment or to *de novo* generation due to the error-prone nature of the HCV polymerase (28, 37). This issue was observed in the filibuvir monotherapy study, where emergence of resistant strains seemed apparent before 3 days in both the 300- and 450-mg twice-daily (BID) dose groups (19). Although there is a slight tendency to overestimate VL decreases, the model predicts VL decreases within about 0.5-$\log_{10}$ units with good confidence.

The NNPolIs in this analysis included four inhibitors of the palm 1 site and four for various thumb sites (Table 3). The model may predict VL decreases better for the palm 1 site than the thumb sites. Two of the less-good predictions are for the VCH-222 (thumb 2) and MK-3281 (thumb 1). But with only 8 compounds included in the analysis, the accuracy of the prediction for each site cannot be assessed with confidence.

For VCH-222, the $\log_{10}$ VL decrease was underestimated by about 2 $\log_{10}$ intervals. One possible explanation for this poor prediction is that VCH-222 could be a substrate for liver uptake transporters, as suggested by accumulation in human hepatocytes as observed with B-CLEAR data (4). The only LPR value for VCH-222 reported to date was low (a value of 5 observed in rats), which could indicate that the rat is not a good model for human hepatic uptake of VCH-222.

To see a wide range of PK/PD response in terms of VL decreases ranging from 1 to >3, the C_{min} range in a study should cover orders of magnitude (Fig. 1 and 2). For one NNPolI, BI 207127, such a C_{min} range was covered in a monotherapy study. BI 207127 could not be used in this analysis because $EC_{50,PS}$ data were not available. But in a BI 207127 monotherapy study in which doses ranged from 100 mg three times a day (TID) to 1,200 mg TID, the difference in C_{min} of the high dose relative to the low dose was about 30-fold, and the median VL decrease was 0.2 $\log_{10}$ IU/ml for the low dose and 3.6 $\log_{10}$ IU/ml for the high dose (31). In compounds included in the current study, the largest C_{min} range covered was for setrobuvir, which had a 3-day monotherapy study including doses of 200, 400, and 800 mg BID, with a difference in C_{min} for the high dose relative to the low dose of about 5.3-fold. However, the range of $C_{min}/EC_{50,PS}$ ratios included in this study was larger than might be expected based on the 5.3-fold difference in C_{min}, due to the ability to assess GT-1a and GT-1b VL decrease data separately. Most of the monotherapy studies for NNPolIs did not cover a wide enough range of exposures to fully characterize the dose-response relationship.

PK/PD relationship for PIs. A summary of monotherapy studies on PIs in chronic HCV GT-1 patients, including the average $\log_{10}$ VL decrease for each dose group, is listed in Table 2. Log_{10} VL decreases of about 4 $\log_{10}$ IU/ml on day 3 of monotherapy were achieved by ABT-450 coadministered with ritonavir, BI 201335, danoprevir, narlaprevir, and vaniprevir. The ratios of C_{min} relative to the EC_{50} or the $EC_{50,PS}$ for the high-dose group, even for compounds with a similar VL decrease, varied widely (Table 5). Unlike NNPolIs, wide ranges of C_{min} values were included in the PI monotherapy studies (e.g., up to a 110-fold range of C_{min} from the low dose relative to the high dose for GS-9256).

LPR values were reported for various PIs in the mouse, rat, dog, monkey, and/or chimpanzee. The lowest LPR reported was about 7 for ABT-450 with and without ritonavir coadministration in dogs (Table 4). This low value of 7 suggests that for ABT-450 in dogs, liver uptake transporters may not have a large effect on liver concentrations, although LPR data should be interpreted with caution, as discussed further in Discussion. Given the good VL decreases achieved by this compound, it is possible that the dog model was not representative of humans in this instance. The highest LPR was for ACH-1625, with a value of 505 determined in rats.

The purpose of this analysis was to determine whether an *in vitro* measure of potency incorporated in the VK model could be used to predict VL decreases. Four PK/PD indices were examined in this context. First, in an approach similar to that used for NNPolIs, $C_{min}/EC_{50,PS}$ was used as model input and the VK model prediction was compared to observed data (Fig. 3a). However, this PK/PD index significantly underestimated VL decreases for PIs. The use of the C_{min}/EC_{50} ratio also resulted in underestimation of VL decreases (Fig. 3b).

PIs often have high LPRs, suggesting that they are often good substrates for liver uptake transporters. These transporters can cause increased liver concentrations. Compounds that are good organic anion-transporting polypeptide (OATP) substrates with low to moderate permeability can accumulate in the liver, as reflected by a high LPR, thereby increasing the free concentration of PI at the target and improving antiviral activity (47). Therefore, PK/PD indices that included the LPR were also examined. The PK/PD index $C_{min} \times LPR/EC_{50,PS}$ still underestimated the $\log_{10}$ VL decrease (Fig. 3c). However, the PK/PD index $C_{min} \times LPR/EC_{50}$ resulted in reasonably accurate predictions of VL decrease (Fig. 3d), predicting VL decreases on day 3 of monotherapy within about 1 $\log_{10}$ in most cases.

In general, increasing C_{min} values led to increasing $\log_{10}$ VL decreases (Fig. 3). To achieve one additional $\log_{10}$ VL decrease, an additional 10-fold increase in C_{min} was generally required, assuming that the LPR was independent of plasma concentrations. The exception was ABT-450 coadministered with ritonavir, for which C_{min} covered a 16-fold range but which had VL decreases independent of C_{min}.

The LPR was found to be important to improve the prediction of VL decreases in this analysis. For the two PK/PD indices that did not include the LPR, $C_{min}/EC_{50,PS}$ and C_{min}/EC_{50}, the values of each index related to high VL decreases spanned about four orders of magnitude (Fig. 3). For the PK/PD index $C_{min} \times LPR/EC_{50}$, there is still a lot of scatter at the highest VL decreases. However, the data are more consistent with the model. Although the $EC_{50,PS}$ relative to the total C_{min} was initially expected to be a more reasonable predictor for PK/PD due to the high protein concentration in the assay, the EC_{50} was the better predictor in the case of the PIs. The LPR data are from a variety of species and study designs. The inconsistent LPR data and the uncertainty as to how the LPR in preclinical species translates to humans could be important reasons for discrepancies remaining between the model and observed data.

DISCUSSION

The current analysis was conducted with the goal of identifying the most important factors that determine the clinical pharmacodynamics of HCV PIs and NNPolIs. A clear relationship between C_{min}, potency, and extent of VL decrease was characterized across

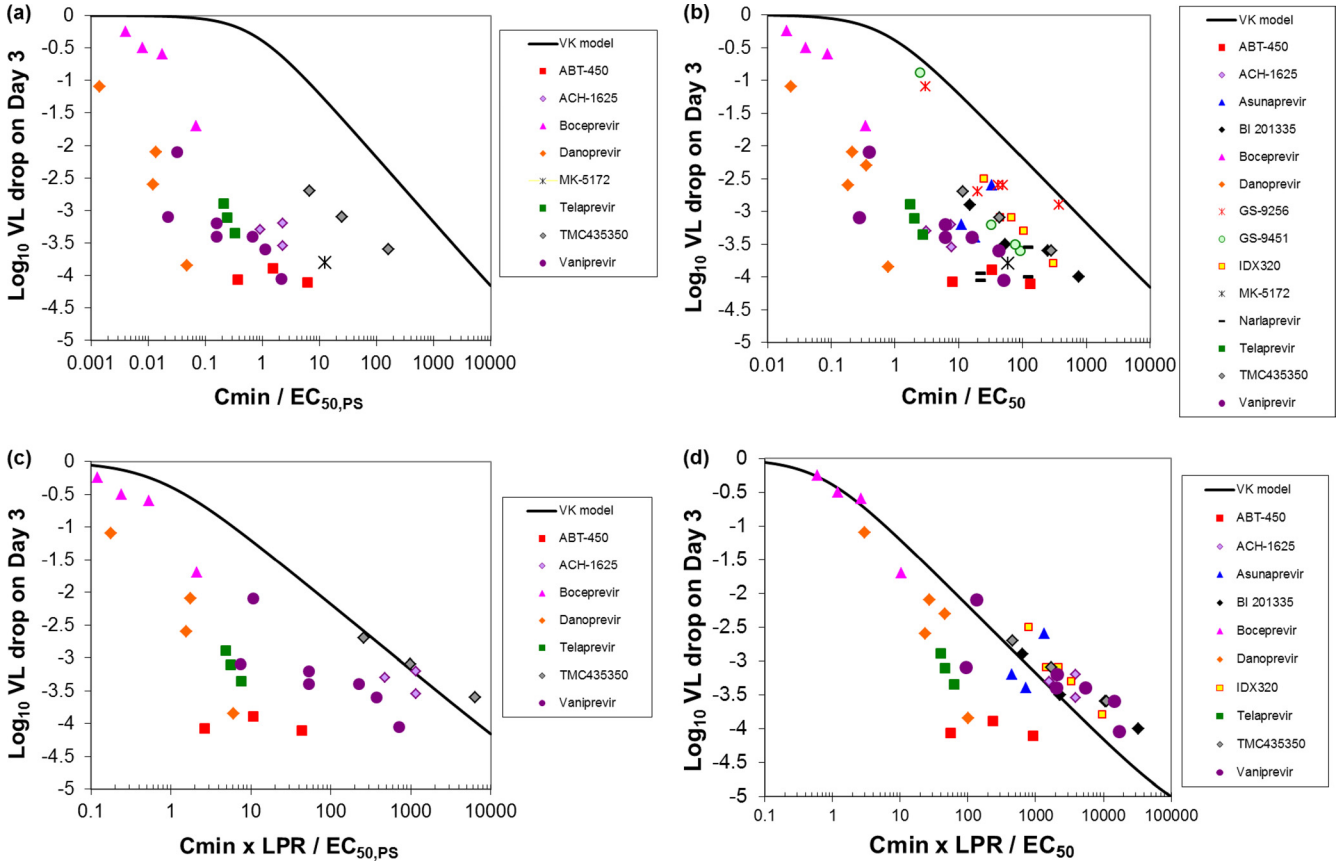

FIG 3 Predicted (curve) and observed (symbols) VL decrease from baseline on day 3 of PI monotherapy ($\log_{10}$ IU/ml) as a function of (a) $C_{min}/EC_{50,PS}$, (b) C_{min}/EC_{50}, (c) $C_{min} \times LPR/EC_{50,PS}$, and (d) $C_{min} \times LPR/EC_{50}$. ABT-450 and two of the four narlaprevir doses were coadministered with ritonavir.

drugs of a common class, and when these factors were incorporated into a model of HCV viral kinetics, the model provided an excellent prediction of clinically observed data across a wide range of studies. Interestingly, for HCV PIs and NNPolIs, different factors related to potency determined the pharmacodynamics in this analysis.

In HIV, the PK/PD of both protease inhibitors and nonnucleoside reverse transcriptase inhibitors are generally similar and, reportedly, often a function of both the plasma C_{min} concentration (although the most appropriate measure of exposure for PD assessment is the subject of some debate) and the EC_{50} of the HIV (29, 49). Similarly, for both HCV PIs and NNPolIs, the combination of C_{min} and potency appear to be important factors driving antiviral activity. However, the PK/PD of HCV compounds appears to be comparatively more complex and must additionally take into account the concentration of drug at the site of action in the liver. For the majority of HCV NNPolIs, active liver uptake does not appear to be an important determinant of liver concentrations, and $EC_{50,PS}$ was adequate to account for unbound drug getting to the site of action. However, for HCV PIs, active liver uptake appears to be common, and thus, the LPR for each compound must be considered a critical factor in predicting its antiviral activity.

Our results suggest that, in general, PIs achieve better clinical potency than the NNPolIs available to date, at least in part due to higher LPRs. As demonstrated by the VK model predictions, NNPolIs with greater potency and/or improved PK should be able to achieve reductions in VL after 3-day monotherapy similar to those observed with PIs. While the higher LPRs contribute to clinical potency differences, we cannot rule out the possibility of fundamental differences in the two targets which may also affect clinical potency. For example, ABT-072 (NNPolI) and ABT-450 (PI) have very similar EC_{50} and $EC_{50,PS}$ values for both HCV GT-1a and -1b (Table 3). ABT-450 reportedly has an LPR of approximately 7 in dogs with or without ritonavir coadministration. However, ABT-450 coadministered with ritonavir achieved a $\log_{10}$ VL decrease from baseline of about −4.1 with a plasma C_{min} of 0.005 μg/ml at day 3, while for ABT-072, a 60-fold higher C_{min} (0.3 μg/ml) resulted in a $\log_{10}$ VL reduction from baseline of about −1.6. As mentioned previously, it is possible that the dog model does not represent the human response in terms of LPR for ABT-450, but target differences also cannot be ruled out.

For PIs, clinical outcomes of patients undergoing treatment in combination with pegylated interferon-ribavirin can differ between HCV GT-1a and -1b, e.g., for boceprevir (3) and danoprevir (62). Differences observed with combination therapy outcomes have been primarily due to differences in the development of resistance between GT-1a and GT-1b. However, in the setting of 3-day monotherapy, there do not appear to be any relevant differences in potency by GT after accounting for the differences in EC_{50}. Although expansion of preexisting mutants can take place early on, at least for some compounds, it seems unlikely that the number of resistant mutations is large enough to contribute such a difference to the measurement of total VL and account for the

outlier compounds in the model. In other words, the same VL reduction in 3 days of monotherapy would be expected for a GT-1a or -1b virus with the same EC_{50}. Therefore, the development of more potent PIs against GT-1a may be less important than the development of newer drugs or drug combinations with a higher barrier to resistance.

Limitations of the analysis. One limitation of the current analysis is in understanding the target concentration for PIs. Here, the LPR was used to incorporate the effect of liver uptake transporters in the model. However, the LPR can also mislead. It is a gross estimate of relative amounts of total compound in the liver and plasma, and determination of liver concentrations does not differentiate between the concentration in the tissue versus the compound that has been excreted into the bile ducts. Some NNPolIs have high LPR values in preclinical species. For example, IDX375 has LPRs greater than about 100 in rats and mice (16) but did not seem to have increased antiviral activity, which suggests that liver uptake transporters are not responsible for the high LPR values. Also, species differences in LPR values can be difficult to interpret and to predict. Finally, the model assumes that the LPR remains relatively constant, but it is possible that the LPR could be a function of time that changes with drug concentration if hepatic transporters become saturated. A more rational, useful approach to using the LPR would be scaling *in vitro* hepatocyte uptake data for identified uptake transporter substrates, as illustrated in reference 56, and using the data for physiologically based pharmacokinetic (PBPK) model development to understand the free concentration in the liver.

The PK/PD approach for this analysis was relatively simple, focusing on C_{min} as the concentration driving VL reduction via an E_{max}-type model. The C_{min} concentration was utilized as it appears for both classes of compounds to be the pharmacodynamically linked variable and a primary determinant of clinical antiviral activity. However, a more sophisticated approach utilizing models that capture the full time course of both PK and PD may have the potential to further improve the observed relationships identified in this analysis, including the ability to predict the antiviral activity of new compounds. In several cases, the full PK profile was tested as an alternative to C_{min}, but this did not significantly change the results for the compounds tested. However, there could be cases where inclusion of the entire PK profile would improve the model. In addition, there could be value in additional exploration of PK/PD indices or other modifications, such as the addition of a Hill coefficient, which could have the potential to further improve the model.

PK differences between HCV patients and healthy volunteers. In HCV patients, as fibrosis progresses into cirrhosis, physiological changes occur that can affect PK. Factors affecting PK in patients with liver disease include differences in cardiac output, hepatic blood flow, number of functional hepatocytes, expression of P450s, albumin level, and renal function, among others (23). Decreased expression of several hepatic P450s and transporters is correlated with the progression of fibrosis (48). For several HCV compounds, significant differences in PK have been observed between healthy volunteers and HCV patients, e.g., for PI TMC435350 (60) and NNPolI IDX375 (9).

For ABT-072 and vaniprevir, PK data in HCV patients were either not available or insufficient to be used in this analysis and the C_{min} values were based on PK in healthy volunteers. For ABT-072, the PK in HCV patients from 2 days of dosing appeared similar to the PK in healthy volunteers from 1 day of dosing, i.e., the half-life ($T_{1/2}$); in healthy volunteers, it was 7.63 $\pm$ 2.32 (mean $\pm$ standard deviation; $n = 8$), and in HCV patients, it was 8.97 $\pm$ 0.20 ($n = 4$) (25). If vaniprevir exposures were higher in HCV patients than healthy volunteers, the prediction of VL decrease would have been less accurate, but the observed trend (an order of magnitude increase in C_{min} resulting in an additional $\log_{10}$ IU/ml decrease in VL) should still be similar.

Considerations in designing a proof-of-concept monotherapy study. The analysis presented here should be considered in the development of a DAA candidate. These results are useful for designing phase 1 monotherapy studies in HCV patients for NNPolIs and PIs and for understanding factors driving VL decreases. The FDA's draft guidance document on developing DAAs for treatment of HCV says that an initial proof-of-concept (phase 1b) monotherapy trial demonstrating initial antiviral activity of the DAA in HCV-infected patients should be conducted as part of an early clinical development program for a DAA candidate. The results from this trial would guide further development, including dose selection, in subsequent phase 2 dose and duration optimization trials in which patients are treated for longer durations (potentially up to 48 weeks) with the DAA as part of a combination regimen (12). Thus, the doses selected for the initial monotherapy trial are critical for informing future longer-term trials. The ability to predict clinical observations enables the identification of clinically meaningful doses for evaluation in the proof-of-concept monotherapy trial and provides a framework for the overall early development of the NNPolI or PI. Thus, the identification of factors that determine the clinical pharmacodynamics of HCV NNPolIs provides valuable information in guiding a 3-day proof-of-concept monotherapy study in HCV patients.

In planning the strategy for drug development, the value of a 1-day monotherapy study as an alternative to the 3-day or longer monotherapy study might be considered for the first study in HCV patients. The generic VK simulation is useful for understanding the utility of a 1-day monotherapy study. As shown in Fig. 1, if an NNPolI has $C_{min} > 100 \times EC_{50,PS}$ or a PI has $C_{min} > 100 \times EC_{50}$/LPR, the VL decrease will be about 2 $\log_{10}$ independent of potency, as illustrated by the overlap of curves for times up to 1 day. If a compound has good potency, at least a 1-$\log_{10}$ VL reduction should be observed. A 1-day monotherapy study can be used to determine whether a compound will have any antiviral activity in HCV GT-1 patients. However, a 1-day study is not expected to differentiate between the activities of potent compounds or to determine whether a compound will have sufficient VL decreases compared to those for drugs on the market or in late-stage development.

Interestingly, although the 3-day proof-of-concept monotherapy study has important implications for the development of a DAA candidate and is used to guide further development of the DAA candidate, including larger phase 2 efficacy and safety trials, there is limited information within the FDA guidance document on this key initial trial, particularly with regard to dose selection. The guidance recommends only that the doses selected for the monotherapy trial should be predicted to provide plasma drug exposures expected to exceed, by severalfold, the protein binding-adjusted cell culture EC_{50} value of the agent and that subsequently these data be used to develop a mechanism-based VK model to guide phase 2 study design (12). For PIs, this strategy may often result in competitive VL decreases. However, for NNPolIs, higher

exposures are probably needed to achieve high VL decreases in monotherapy. Although the value of the NNPolI might be mainly due to its antiviral activity on PI-resistant strains, a good VL decrease is still more desirable than a poor VL decrease. The results here suggest that for an NNPolI to achieve a 3-$\log_{10}$ VL decrease, $C_{min} > 700 \times EC_{50,PS}$ must be achieved. Therefore, good biopharmaceutical properties that allow high exposures in patients are desirable. Even for PIs, achieving high exposures helps to achieve high VL decreases, although there is more variability in how high the exposures have to be. For example, danoprevir resulted in a decrease of about 3.9 $\log_{10}$ IU/ml in VL within 3 days of monotherapy with a C_{min} of 0.001 μg/ml, while TMC435350 achieved a decrease in VL of 3.6 $\log_{10}$ IU/ml with a C_{min} of 4.8 μg/ml (Table 2).

The novel PK/PD approach presented here for the prediction of declines in VL incorporates the FDA-recommended consideration of the VK model but in an earlier application, and it leverages the immense available *in vitro*, preclinical, and clinical data for a range of NNPolIs and PIs in development to identify relationships between *in vitro* potency values, liver-to-plasma distributions (for PIs), and clinical PK/PD data to identify factors involved in clinical pharmacodynamics of NNPolIs and PIs and to characterize the VK-PK/PD relationship for two classes of DAAs. As practicality may limit the range of doses to be tested in the phase 1b trial, combining data from multiple compounds allows a broader range of exposures relative to potency to be evaluated and thus enables efficient identification of clinically meaningful doses for evaluation. Indeed, many individual studies did not cover a wide enough range of C_{min} to fully characterize the dose-response relationship (Table 5); however, combining data for multiple competitor compounds elucidates the relationship.

For a given dose and the resulting C_{min}, the predicted VL decrease can be expected to be within about 0.5 to 1 $\log_{10}$ of the observed value (Fig. 2, Fig. 3). This approach therefore enables the prediction of efficacious exposures and, through further physiologically based PK-modeling techniques, aids in the identification of appropriate doses and dose ranges to be evaluated in the clinical trial. Though this VK-PK/PD approach has demonstrated excellent prediction of clinically observed data across a wide range of studies, one must consider the uncertainty inherent in the predictions. It is noted that for PIs, there are several outliers that perform significantly better than the prediction, as the observed 4-log VL decrease values of $C_{min} \times LPR/EC_{50}$ span three orders of magnitude (Fig. 3d). It appears that the PI model is particularly good for identifying less active compounds but could have the potential, if too rigorously applied, to improperly characterize some of the most potent compounds (e.g., danoprevir and ABT-450) as poor candidates. For these compounds, it is possible that the LPR is greater than the estimate, or there could be additional factors contributing to the antiviral activity that are not captured in the model.

The results here are specifically for NNPolIs and PIs but provide a framework that is generally applicable for anti-HCV drugs. Compounds that are not liver uptake transporter substrates may be expected to behave like NNPolIs, while compounds that are liver uptake transporter substrates may be expected to behave like PIs. Assessing model predictions against available clinical data from monotherapy studies for DAAs in clinical development for a given target should be done to gain confidence in this approach before applying it to new targets besides NNPolIs and PIs.

Conclusions. The results presented here indicate a difference in PK/PD behavior for NNPolIs and PIs. For NNPolIs, using $C_{min}/EC_{50,PS}$ as input to the VK model results in good prediction of VL decrease. For PIs, $C_{min} \times LPR/EC_{50}$ is the PK/PD index that results in good prediction of VL decreases.

APPENDIX

For one-compartment PK, the steady-state trough concentration, $C_{min,SS}$, after n doses, $C_{min,n}$, can be estimated as follows (70):

$$C_{min,n} = \frac{D}{V} \times \frac{1 - \exp(-0.693n\,\tau/T_{1/2})}{1 - \exp(-0.693\tau/T_{1/2})} \times \exp(-0.693\tau/T_{1/2}) \quad \text{(A1)}$$

where D is the dose, V is the volume of distribution, τ is the dosing interval (e.g., 12 h for BID administration), and $T_{1/2}$ is the half-life. When the concentration at the end of the first dosing interval, $C_{min,1}$, and $T_{1/2}$ are available from a single-dose PK study, $C_{min,n}$ can be approximated using the following equation derived using equation A1:

$$C_{min,n} = C_{min,1} \times \frac{1 - \exp(-0.693n\,\tau/T_{1/2})}{1 - \exp(-0.693\tau/T_{1/2})} \quad \text{(A2)}$$

The following simplified equation can be used to estimate $C_{min,SS}$ if steady state was achieved by day 3:

$$C_{min,ss} = C_{min,1} \times \frac{1}{1 - \exp(-0.693\tau/T_{1/2})} \quad \text{(A3)}$$

Assuming that it takes about 4 to 5 $T_{1/2}$ intervals to achieve steady state, if $T_{1/2}$ is less than about 14 to 18 h, equation A3 can be applied.

Monotherapy studies were often conducted for more than 3 days, and the C_{min} value on the last day of the study was reported (e.g., for danoprevir). In such cases, if $T_{1/2}$ was less than 18 h, it was assumed that steady state had been achieved by day 3 and that the C_{min} on day 3 was the same value as the C_{min} at the end of the study.

For some compounds, additional PK calculations were needed, as follows.

For ABT-072, the mean dose-normalized C_{min} for once-daily administration for the 400- and 800-mg doses was used to estimate the day 3 C_{min} for different doses, assuming dose-proportional PK, which had been observed for single doses up to 1,200 mg.

For ABT-333, the C_{min} data were from a different study than the VL decrease data, which did not report PK. In the study that reported PK (26), ABT-333 was administered in 2 days of monotherapy followed by 26 days of coadministration with pegylated interferon and ribavirin. The C_{min} values were similar on days 2, 3, 4, and 5 for both the 300- and 600-mg BID doses. It was assumed that due to dose-limited absorption, the C_{min} for the 600-mg BID dose could be used to estimate the C_{min} values for the 400- and 800-mg BID doses in the other study.

For IDX375, C_{min} values were not reported (21); the PK for 200-mg BID dosing (9) were used to estimate C_{min} after repeat dosing using equation A2 and assuming dose-proportional PK, which had been observed. This method was used because the $T_{1/2}$ for IDX375 in HCV patients was 36.4 h.

For tegobuvir, single-dose PK data were reported (1). The C_{min} on day 3 was calculated with equation A3.

For boceprevir, $C_{min,SS}$ values reported in a GT-2 and -3 study for doses of 200 mg BID, 400 mg BID, and 400 mg TID (65) were used to estimate C_{min} values for the GT-1 studies, since the C_{min} values were not reported in the GT-1 studies. The $C_{min,SS}$ was estimated as the average of the reported C_{min} values on day 11, 12,

13, and 14. It was assumed that the day 3 C_{min} would be about the same as the $C_{min,SS}$ because the $T_{1/2}$ was about 8 h. The 200-mg TID C_{min} was estimated from the 400-mg TID value, and the 100-mg BID C_{min} was estimated from the 200-mg BID value, assuming dose-proportional PK.

For asunaprevir, only single-dose PK data were plotted in the monotherapy report (51). The C_{min} data used in this analysis were steady-state values from a combination study with a 600-mg BID dose; the mean from two groups, asunaprevir with BMS-790052 or BMS-790052, pegylated interferon, and ribavirin, was used (41). To estimate C_{min} for the other dose levels, linear PK were assumed. Although $T_{1/2}$ was not reported, it was assumed that $C_{min,SS}$ could be used because of the claim that the compound PK supported BID administration, which suggests that $T_{1/2}$ was less than 18 h.

For narlaprevir, the C_{min} data used in this analysis were steady-state values from a combination study with peginterferon alpha-2b (58). It was assumed that $C_{min,SS}$ could be used because for the BID regimen (i.e., administered with ritonavir), the $T_{1/2}$ was about 16 h, and for the TID regimen (i.e., no ritonavir), the $T_{1/2}$ was about 5 h.

For BI 201335, the $C_{min,SS}$ on day 28 with coadministration with pegylated interferon alpha-ribavirin was reported. The day 3 C_{min} was estimated by combining equations A3 and A2. The $T_{1/2}$ of BI 201335 was estimated as 27.6 h, the average value for the four dose groups. This method might overestimate C_{min} somewhat since the PK were greater than dose proportional.

For telaprevir, it was assumed that the C_{min} on day 3 would be similar to the reported $C_{min,SS}$ since the $T_{1/2}$ was about 4 h at doses of 450 mg and higher (6).

For TMC435350, the day 3 C_{min} was reported for the study that included the 200-mg dose (60). Only $C_{min,SS}$ was reported for the study that included 25- and 75-mg doses (43). Nonlinear clearance apparently affected the 200-mg dose, which had about 20-fold higher AUC values than the 75-mg dose. Therefore, although the $T_{1/2}$ for the 200-mg dose was about 41 h, it was assumed that the $T_{1/2}$ intervals for the 25- and 75-mg doses were more like those of healthy volunteers (i.e., about 10 h) and that, for those two doses, the day 3 C_{min} could be approximated as the $C_{min,SS}$.

For vaniprevir, PK was reported to be nonlinear, with slightly greater than dose-proportional increases in PK (i.e., AUC, C_{max}, and concentration in plasma at 12 h) with increasing doses. The exposures achieved in the monotherapy study were not reported. In the PK study with healthy volunteers, the doses and regimens were not the same as included in the monotherapy study. The day 3 C_{min} was based on the C_{min} of the closest dose, assuming dose-proportional kinetics, and then adjusted as necessary to reflect a $C_{min,SS}$, which was assumed to represent the day 3 C_{min} because the $T_{1/2}$ was about 4 to 5 h following multiple oral doses (73). For example, the 25-mg BID day 3 C_{min} was estimated based on the 40-mg PK, adjusting for the different dose by assuming dose-proportional PK between these similar doses and the effect of repeat exposure using equation A3. At the 40-mg dose, only the C_p at 6 h was reported, presumably from detection limit problems, and so the C_p at 12 h was adjusted for the expected decrease over 6 h.

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